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Modulation of Microglial Pro-inflammatory and Neurotoxic Activity for the Treatment of Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is a debilitating movement disorder resulting from a progressive degeneration of the nigrostriatal dopaminergic pathway and depletion of neurotransmitter dopamine in the striatum. Molecular cloning studies have identified nearly a dozen genes or loci that are associated with small clusters of mostly early onset and genetic forms of PD. The etiology of the vast majority of PD cases remains unknown, and the precise molecular and biochemical processes governing the selective and progressive degeneration of the nigrostriatal dopaminergic pathway are poorly understood. Current drug therapies for PD are symptomatic and appear to bear little effect on the progressive neurodegenerative process. Studies of postmortem PD brains and various cellular and animal models of PD in the last 2 decades strongly suggest that the generation of pro-inflammatory and neurotoxic factors by the resident brain immune cells, microglia, plays a prominent role in mediating the progressive neurodegenerative process. This review discusses literature supporting the possibility of modulating the activity of microglia as a neuroprotective strategy for the treatment of PD.

KEYWORDS: Dopamine neuron, Parkinson's disease, movement disorder, microglia, neuroprotection, free radical

INTRODUCTION

Parkinson's disease (PD) was named by French neurologist Jean-Martin Charcot after the British physician James Parkinson, who in 1816 first described the disease in his publication entitled "An Essay on the Shaking Palsy."[1,2] The clinical symptoms of PD include slow movement, resting tremor, rigidity, and posture instability. Pathologically, the development of clinically identifiable PD symptoms results from a massive loss of the dopaminergic neurons in the substantia nigra (SN) and a marked reduction in the release of the neurotransmitter, dopamine (DA), by those SN dopaminergic neurons that project into the neostriatum.[3,4] It is generally accepted that the appearance of currently identifiable clinical symptoms requires the loss of more than 50% of the DA-producing and DA-releasing capacity by the nigrostriatal dopaminergic pathway.

Genetic and molecular cloning studies of mostly familial clusters of PD cases have associated certain early-onset PD with mutations in several genes or loci. The proteins that some of those genes encode include parkin, alpha-synuclein, PTEN-induced kinase 1, DJ-1, ubiquitin carboxyl-terminal esterase L1, and leucine-rich repeat kinase 2.[5] Studies of the genetic forms of PD have offered important insight into the pathogenetic process of the disease as well as model systems to test potential intervention strategies.[6] However, genetic forms of PD only represent a small fraction (<5%) of the total PD cases. The cause(s) of the majority of PD cases remains undefined.

Lack of a clear understanding of its pathogenetic process at the molecular level and of its etiology for most cases has certainly contributed to our current deficiency in effective neuroprotective strategies to slow, or better yet, to halt the progressive degeneration in PD. Current drug therapies for PD with the dopamine biosynthesis precursor levodopa or various DA receptor agonists are for symptomatic management, and they appear to bear little effect on the neurodegenerative process.

Idiopathic PD is an age-related disease with a medium onset age of 60. The incidence of PD rapidly increases with age. In the United States, PD is the second most common degenerative neurological disorder and currently affects approximately 1 million people. With the first wave of nearly 80 million baby boomers in the United States entering their sixth decade of life, the incidence of PD is expected to rise significantly, making it even more urgent to find a cure for the disease. Worldwide, contrary to earlier estimates, the prevalence of PD in the developing countries does not appear to be vastly lower than that in the developed countries.[7-9] A rapid expansion of the aged population, increased awareness in the general public, and more accurate clinical diagnosis have been attributed to the seeming "rise" in the prevalence of PD in the developing countries. Hence, the aging of the

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entire population of the world certainly presents a global challenge to the scientific community to find a cure for PD.

Microglial Activation and Analysis of Postmortem PD Brains

Microglia, historically also known as the "Hortega cells" because of the initial and detailed description by Spanish neuropathologist del Rio-Hortega, are the resident immune cells in the brain.[10] The phagocytic capacity of microglia is thought to be important in the brain remodeling process during early development, as well as injury repair. In addition, microglia possess certain characteristics of antigen-presenting cells. As the resident immune cells in the brain, microglia are particularly sensitive to pathological changes in the brain. They rapidly become activated in response to invading pathogens, the presence of foreign substances, or neuronal injuries inflicted by trauma, ischemia, axotomy, or neurodegeneration.[11-13] Activation of microglia is characterized by dramatic morphological changes, increased expression of surface molecules, and secretion of a wide variety of soluble factors that include cytokines, oxygen and nitrogen free radicals, chemokines, fatty acid metabolites such as prostaglandins, and trophic factors.

The involvement of microglial activity in the pathogenesis of PD perhaps was initially proposed following the observation of reactive microglia in the SN of postmortem brains of PD patients.[14] Subsequent studies from other groups reported the presence of soluble factors and enzymes indicative of the occurrence of inflammation in the SN. These include pro-inflammatory cytokines tumor necrosis factor-alpha (TNFα), interleukin-1 beta (IL-1β), IL-2, IL-6, and interferon gamma (IFNγ), as well as inducible nitric oxide synthase (iNOS).[15]

The discovery of reactive microglia and various pro-inflammatory factors in the SN of terminal stage PD brains per se was initially considered to be simply the consequence of a microglial reactive response toward the massive loss of neurons. Under this assumption, microglial activation, at least at the degree that can be detected at the moment, was thought to occur at a rather late stage of the disease process. Hence, the involvement of microglia was considered to be fairly passive and to have little effect on the dynamic neurodegenerative process. However, findings in the last decade from epidemiological studies, various animal PD models, and cell culture studies have provided rather compelling evidence in support of an active role for microglia at a rather early stage of the progression of PD.

Nonsteroidal Anti-inflammatory Drug Use and Incidence of PD

Cyclooxygenase (COX) is one of the key enzymes involved in the production of pro-inflammatory lipid mediators such as prostaglandins in various types of cells including microglia. Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective inhibitors of COX enzymes that block the production of prostaglandins. Several epidemiological studies of large cohorts determined the potential effect of NSAID use on the incidence of PD. In one study, large cohorts (44 000 male and 98 000 female) of middle-aged to old-aged healthy individuals in the United States were followed for more than 10 years, and the incidence of PD was compared between regular and non-regular users of NSAIDs. A significantly lower (up to 45%) incidence of PD was observed in regular users of nonaspirin NSAIDs compared with non-regular users. A similar degree of reduction in PD incidence was observed in both male and female participants.[16] In a subsequent study that confirmed the reduced risk of PD in NSAID users, the regular use of ibuprofen, but not other nonaspirin NSAIDs was associated with the reduction in PD incidence.[17] Another report of a case-control study based on a different set of participants found a lower risk for PD only in male, nonaspirin NSAID users.[18] These findings suggest that inhibition of the production of pro-inflammatory lipid mediators modifies the disease progression process for PD.

Temporal Relationship Between Microglial Activation and DA Neurodegeneration in Experimental PD Models

Besides correlative evidence based on analysis of postmortem PD brains and epidemiological cohorts, more direct evidence in support of an active role for microglia in PD pathogenesis has come from various experimental PD models. In most cases, the realization that glial activation is an integral component of the pathogenetic process, at least in the PD models studied, has "revised" our understanding of how the toxicants induce dopaminergic neurodegeneration (Figure 1). In other cases, activators of glial cells were applied, at least as a proof of principle, to induce neurodegeneration. Overall, these studies have at least helped "divide" the effort of PD research between focusing purely on the death of DA neurons and paying closer attention to the roles of nonneuronal cells (ie, glia) in order to fully understand the disease and to devise effective neuroprotective strategies. Summarized in Table 1 are key findings from various experimental PD models that made possible the construction of a temporal relationship between microglial activation and DA neurodegeneration.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine PD model

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a by-product of an illicit drug synthesis scheme, was found to

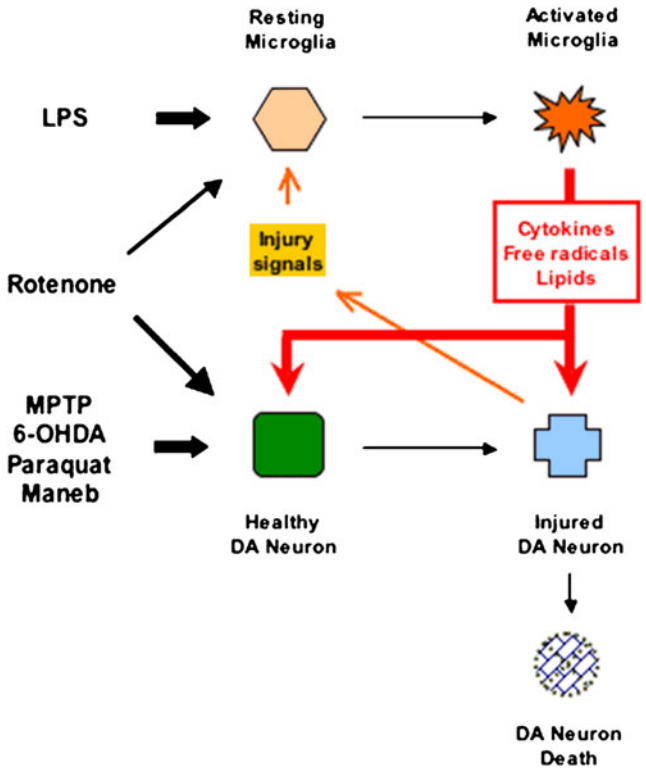

Figure 1. Schematic representation of the involvement of microglia in the dopaminergic neurodegenerative process induced by various agents. LPS indicates lipopolysaccharide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; and DA, dopamine.

cause PD in accidentally dosed humans in the 1980s.[39,40] Incidentally, MPTP has become the most widely used agent to reproduce PD pathologies and behavioral abnormalities in mice and nonhuman primates.

In several earlier studies designed to determine the temporal relationship between DA neurodegeneration and glial activation, significant degeneration of striatal dopaminergic fibers occurred 2 days after MPTP dosing.[19,20] Activation of astroglia in the striatum was detected between 24 and 48 hours post-MPTP dosing. The duration of the astroglial activation appeared to be dependent on the degree of initial DA damage, where sustained activation was observed throughout the study in mice injected with 4 doses of MPTP in 1 day (6 weeks).[20] Mechanistically, blockade of MPTP-induced DA lesions prevented the occurrence of astroglial activation.[19] Microglial activation, on the other hand, was examined at 1 time point (48 hours), and robust activation was observed.

In later studies, where the temporal relationship between microglial activation and DA lesions was more closely examined, microglial activation in the SN was detected as early as 1 day after MPTP dosing and sustained for 14 days. Astroglial activation trailed microglial activation by 24 hours. Although signs for DA lesions appeared early, significant SN DA neuron loss did not become apparent until 7 days after MPTP administration.[21,22] In even more detailed time-course studies, microglial activation was rapid (detected as early as 12 hours and peaked at 1 day after MPTP dosing) yet transient (subsided to control level in about a week).[23,24] Astroglial activation, on the other hand, peaked at day 4 and did not return to control level till the end of the study (day 21).[23] In most recent studies using highly sensitive real time polymerase chain reactions to detect the expression of glial activation-associated genes, significant upregulation of genes for IL-1α and TNFβ was observed as early as 2 to 4 hours in the striatum after MPTP dosing, preceding striatal dopaminergic neurodegeneration (Figure 2A).[25,26] Collectively, these studies strongly suggest that microglial activation occurs at a very early stage of MPTP-induced DA lesion, responding to the slightest neuronal damages inflicted by MPTP (Figure 1).

6-Hydroxydopamine PD Model

Analogous to the discovery of microglial involvement in MPTP PD models, microglial activation was noticed early on in 6-Hydroxydopamine (6-OHDA)-lesioned rats. In one study, microglial activation appeared as early as 1 day after 6-OHDA dosing but became transient in nature. Astroglial activation, on the other hand, trailed microglial activation but remained sustained.[27] A sustained astroglial activation was also observed in another study.[28] In a recent study using positron emission tomography to detect dopamine neuron- and microglia-specific ligands, a progressive DA neurodegeneration and a concomitant rise in microglial activation were observed. In this case, microglial activation was not transient but remained prominent throughout the entire course of DA neurodegeneration (28 days, Figure 2A).[29]

Lipopolysaccharide PD model

Exposure to pathogens such as viruses and bacteria has been suspected to be a risk factor for the development of sporadic PD.[15,41] Lipopolysaccharide (LPS), a bacterial endotoxin from the Gram-negative bacterial cell wall, is a potent stimulator of immune cells. Administration of LPS to rodent brains has reproduced certain features of PD including a progressive degeneration of the nigrostriatal DA pathway and movement abnormalities. More important, the LPS PD model has served as a powerful and valuable tool to decipher the role of glial cells, especially that of microglia in the DA neurodegeneration process.[17]

A single injection of LPS (2-2.5 μg) to an area slightly above the SN (supranigral region) of the adult rat brain resulted in a rapid activation of microglia followed by a time- and LPS-dose dependent degeneration of the nigrostriatal

Table 1. Glial Activity and Dopaminergic Neurodegeneration in Experimental Animal PD Models*

Toxicant	Animal	Site of DA Neurodegeneration (Range in time)†	Microglial Activation (Range in time)‡	Astroglial Activation (Range in time)	Key References Cited
MPTP	Mouse	ST (day 2)	Yes (day 2)	Yes (day 1-week 2/6)	19,20
	Mouse	SN (day 7, 14, 21)	Yes (SN: days 1-14)	Yes (days 2 and on) (ST: days 1-4)	21,22
	Mouse	SN (day 7) ST (day 7)	Yes (SN: days 0.5-7) (ST: days 0.25 and on)	Yes (days 2-21)	23,24
	Mouse	ST (hour 24, 48)	Yes (ST: hours 2-24)		25,26
6-OHDA	Rat	SN/ST (weeks 2-12)	Yes (SN/ST: days 1-10)	Yes (SN/ST: weeks 1-12)	27,28
	Rat	SN/ST (days 3-28)	Yes (SN/ST: days 3-28)	Yes (SN/ST: days 3-28)	29
LPS	Rat	SN/ST (days 2/3-21)	Yes (SN/ST: hour 6-day 21)		30,31
	Rat	SN (weeks 2/4-10/12)	Yes (SN: day 3-week 12)		32,33
Rotenone	Rat	SN/ST	Yes (SN)		34,35
Paraquat ± Maneb	Rat	ST		Yes (ST)	36
Paraquat	Rat/Mouse	SN/ST	Yes (SN)	Yes (SN)	37,38

*PD indicates Parkinson's disease; DA, dopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ST, striatum; SN, substantia nigra; 6-OHDA, 6-hydroxydopamine; and LPS, lipopolysaccharide.
†Time with the earliest significant change.
‡Range of time starting with the earliest significant change and the last time point with significant changes.

DA pathway.[30,31] In this case, the earliest sign of microglial activation in the SN appeared 6 hours after the LPS injection and reached a maximum at 1 to 2 days.[31] In contrast, significant degeneration of the nigrostriatal DA pathway did not occur until 2 to 3 days later.[30,31] In the single injection LPS PD model, a robust microglial activation and massive loss of DA neurons occurred in the first several weeks after the single LPS injection.[30,31,42] DA neurodegeneration seemed to reach a plateau between 1 and 3 months and did not progress further for up to 12 months after the LPS injection.[43] The plateau arrived earlier if a higher amount of LPS had been injected.[44] Concomitant to the reaching of a plateau for DA neurodegeneration, microglial activation started to gradually subside and, toward the end, was not particular obvious.[43,44] This gradual decline in microglial activation in the single LPS injection model was most likely because of the lack of continuing neurodegeneration that was needed to provide a constant source of fuel (ie, DA neurodegeneration) to sustain a continuing microglial activation (Figure 2B).

To model the effect of chronic microglial activation on the nigrostriatal DA pathway, nanogram quantities of LPS were supranigrally infused, over a period of 2 weeks into the rat brain.[32] In this case significant microglial activation was detected 3 days (the earliest time point examined) after the start of the continuous LPS infusion. Significant loss of SN DA neurons, however, was not detected until 4 weeks after the start of LPS infusion. Microglial activation peaked 1 to 2 weeks after the start of LPS infusion and remained prominent at the 8-week time point (the longest examined for microglial activation). SN DA neuron loss, in the mean time, occurred in a progressive manner and reached maximum at the end of the study (10 weeks).[32]

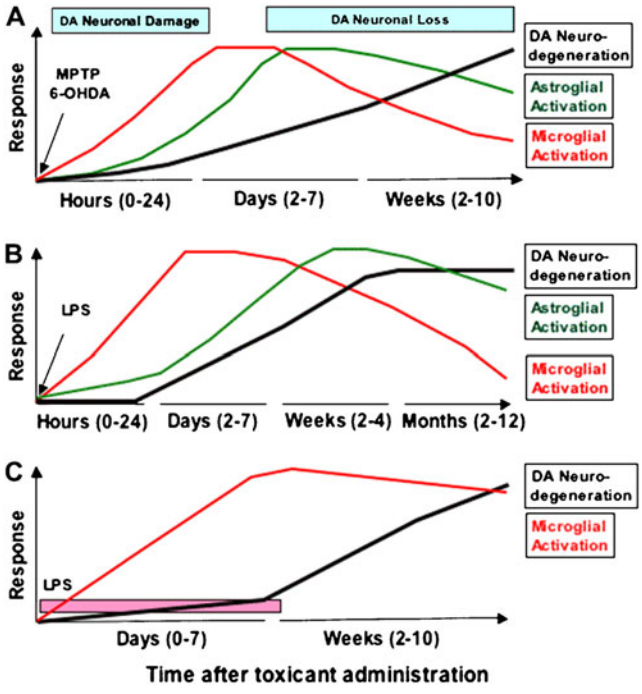

Figure 2. Schematic representation of the temporal relationship between the activation of microglia, astroglia, and dopaminergic neurodegeneration in various Parkinson's disease animal models. DA indicates dopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; and LPS, lipopolysaccharide.

LPS chronic infusion-induced progressive loss of SN DA neurons and sustained microglial activation in the SN were also observed in rats that had been exposed prenatally to LPS and were born with a reduced number of SN DA neurons (Figure 3C).[33]

Rotenone PD Model

Chronic exposure of rodents to rotenone, a pesticide and mitochondrial complex I inhibitor, has been shown to cause DA neurodegeneration, inclusion formation, and motor abnormalities.[45-47] Robust microglial activation was observed in both the striatum and SN of rotenone-treated rats and microglial activation appeared to occur before the occurrence of apparent neurodegeneration.[34] In contrast, astroglial activation seemed to be rather mild and limited in the rotenone-lesioned rats.[34] In a separate study, microglial activation was limited to the striatum of rats that had dopaminergic lesions. Animals that did not have apparent DA neurodegeneration did not exhibit significant microglial activation.[35] Further studies may be needed to determine whether rotenone, in vivo, has the dual function of directly activating microglia and injuring DA neurons as has been observed in cell culture studies.[48]

Paraquat or Paraquat/Maneb PD Model

The herbicide paraquat, either alone or in combination with the fungicide maneb, causes the degeneration of the nigrostriatal DA pathway and deficiency in locomotor activities in rodents.[36-38,49,50] In one study, limited and mild astroglial activation was observed in the striatum of rats treated with paraquat and maneb.[36] In studies using paraquat alone, significant activation of both microglia and astroglia was observed before the apparent loss of SN DA neurons.[37,38] Analogous to the rotenone PD animal model, additional animal studies will be needed to determine the precise role that microglia play in DA neurotoxicity induced by paraquat and/or maneb.[51-54]

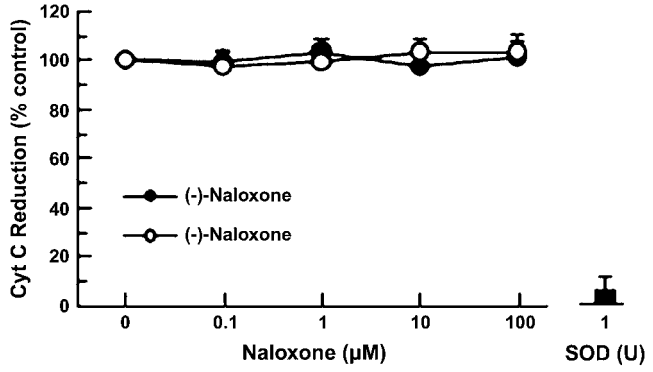

Figure 3. Lack of antioxidant activity for naloxone isomers as determined by the xanthine-xanthine oxidase model system. Assays were performed in the presence of indicated am ounts of naloxone isomers, 0.01 U xanthine oxidase, 50 μM xanthine, 20 μM partially acetylated cytochrome c (cytC) in 50 mM potassium phosphate buffer (pH 7.6) in a 96-well plate (100 μL/well final volume). Xanthine was added to initiate the reaction, and absorbance at 450 nm was continuously monitored for 5 minutes using a Synergy HT multi-detection microplate reader (Bio-Tek Instruments, Winooski, VT). Results were expressed as a percentage of the control and are mean ± SEM of 3 determinations performed in duplicate. Superoxide dismutase (SOD) was used as a control to demonstrate its effectiveness to remove superoxide from the system.

One of the strongest lines of evidence supporting the view that microglial activation is a key driving force of the progressive DA neurodegeneration in PD may have come from several recent studies of MPTP-intoxicated humans and nonhuman primates. In individuals who developed PD after early life MPTP intoxication, robust microglial activation in the SN was visible years after the initial MPTP administration.[55] Sustained microglial activation was also found in the SN of monkeys long after the termination of MPTP injections.[56,57] The discovery of intertwined occurrence of SN DA neuronal loss and microglial activation suggests the existence of a self-amplifying mechanism that promotes the progressive neurodegenerative process in PD.

Detection of Pro-inflammatory and Neurotoxic Factors in Postmortem PD Brains and Experimental PD Models

Microglia readily become activated in response to environmental changes such as the presence of invading pathogens, the occurrence of injuries, and the disturbance in homeostasis.[11-13] Upon activation, microglia undergo dramatic morphological changes: metamorphosing from the ramified resting state to the amoeboid activated state. In addition to morphological changes, activation of microglia results in the upregulation of surface molecules.[58,59] Furthermore, activation of microglia leads to an increase in the production of cytokines such as TNFα IL-1β, IL-6, the induction of iNOS and COX-2, and the activation of enzymes such as NADPH oxidase.

Following the discovery of activated microglia in the SN of postmortem PD brains,[14] various groups have discovered the presence of a panel of microglia-derived factors and activation-associated enzymes in the SN, striatum, and cerebrospinal fluid of PD brains (Table 2). These included cytokines IL-1β,[60-63] IL-2,[60,61] IL-4,[61] IL-6,[60-62,64] TNFα[63,65], IFN,[63] iNOS,[66,67] COX-1,[67] and COX-2.[67] Direct evidence for the activation of NADPH oxidase, the primary enzyme responsible for the production of reactive oxygen species (ROS) in microglia in the SN of postmortem PD brain is lacking. However, multiple lines of evidence exist in support of the notion that oxidative stress

Table 2. Upregulation of Microglial Activation-associated Pro-inflammatory and Neurotoxic Factors and Related Enzymes in Human PD Brains and Experimental Animal PD Models*

Factors / Enzymes	Human PD Brain	MPTP Model	6-OHDA Model	LPS Model
IL-1β	Yes	Yes	Yes	Yes
IL-2	Yes	?	?	?
IL-4	Yes	?	?	?
IL-6	Yes	Yes	?	Yes
TNFα	Yes	Yes	Yes	Yes
IFNγ	Yes	Yes	?	?
iNOS	Yes	Yes	Yes	Yes
COX-1	Yes	?	?	?
COX-2	Yes	Yes	?	?
NADPH oxidase	Yes†	Yes	?	Yes

*PD indicates Parkinson's disease, MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine; LPS, lipopolysaccharide; IL, interleukin; TNFα, tumor necrosis factor-alpha; IFNγ, interferon gamma; iNOS, inducible nitric oxide synthase, and COX, cyclooxygenase.
†Direct evidence is lacking but supporting evidence is abundant (refer to section Detection of Pro-inflammatory and Neurotoxic Factors in Postmortem PD Brains and Experimental PD Models).

plays an important role in the DA neurodegeneration process in PD. First, as a result of elevated oxidative stress, increased lipid peroxidation has been detected in the SN of PD brains.[68-71] Second, the SN of PD brains has an elevated level of iron that may promote ROS formation through the Fenton reaction.[72,73] Third, the SN of PD brains has a reduced level of antioxidant enzymes such as catalase and peroxidase and antioxidant molecules such as glutathione, suggesting the presence of a sustained burden of oxidative stress that has overwhelmed the antioxidant capacity.[74-77] Fourth, SN DA neurons are known to have a defect in the complex I of the mitochondrial electron transport chain, possibly a consequence of oxidative damage of key components of the complex.[78,79] A reduced activity of the mitochondrial respiratory chain may lead to the further elevation of free radical production, the lowering of the threshold for the induction of neuronal death, or both.[80,81]

Aside from postmortem PD brains, increased cytokine expression and activation of enzymes associated with microglial activation have also been detected in midbrains (SN and striatum) of various experimental animal PD models (Table 2). In the MPTP PD model, immunohistochemical, biochemical, and gene array studies have uncovered increased expression of proteins, messages, or both for IL-1β,[82-86] IL-6,[25,82,84,85] TNFα,[25,82-85] iNOS,[23,24,83,84,86] COX-2,[87-89] and subunits of the NADPH oxidase complex.[86,90,91] In the 6-OHDA PD model, increased expression has been detected for IL-1β,[92] TNFα,[93,94] and iNOS.[95,96] In the LPS PD model, upregulation of IL-1β,[97] IL-6,[97] TNFα,[97,98] iNOS,[97,99,100] and NADPH oxidase subunits[44] has been observed. In vitro and in vivo experiments have demonstrated that LPS, a potent stimulator of microglia, induces microglia to secrete cytokines (IL-1β and TNFα), upregulate iNOS to produce nitric oxide, and activate NADPH oxidase to produce superoxide free radical.[15,101] The detection of these factors and enzymes in these PD models, especially at the earlier time points, often before the occurrence of apparent DA neurodegeneration, strongly suggests that these microglia-derived factors contribute to the early phase of the progressive DA neurodegenerative process.

Association of Individual Pro-inflammatory and Neurotoxic Factors and/or Enzymes With Nigrostriatal DA Neurodegeneration in Experimental PD Models

Several approaches have been taken to determine the contribution to the degeneration of the nigrostriatal DA pathway of individual pro-inflammatory and neurotoxic factors and/or enzymes whose upregulation has been observed in PD brains. For individual enzymes of interest, the effects of pharmacological inhibitors on DA neurodegeneration have been tested. A more definitive association has been made by comparing the vulnerability of the nigrostriatal DA pathway of wild type animals with that of gene knockout animals. The contribution of individual cytokines to DA neurodegeneration has been determined with wild type animals and animals with the gene for the cytokine or its receptor(s) knocked out (Table 3).

Cytokines

Mice null for both receptors of TNFα (TNFR1 and TNFR2) were found to be resistant to the MPTP-induced striatal DA neurotoxicity and the activation of microglia and astroglia.[26,102] Deletion of both TNF receptors was necessary to

Table 3. Consequence of Inhibition of Individual Microglial Activation-associated Pro-inflammatory and Neurotoxic Factors and/or Related Enzymes in Experimental PD Models*

Target	PD Model	Inhibitor	Gene Deleted	Consequence
TNFα	MPTP		TNFR1 and 2	Reduced striatal DA depletion and microgliosis
TNFα	MPTP		TNFα	Reduced striatal DA depletion
iNOS	MPTP		iNOS	Reduced loss of SN DA neurons
nNOS	MPTP	7-NI		Reduced loss of SN DA neurons and striatal DA depletion
NOS	6-OHDA	L-NAME		Reduced striatal DA depletion and rotation
iNOS	LPS	S-MITU		Reduced loss of SN DA neurons and rotation
COX-2	MPTP		COX-2	Reduced loss of SN DA neurons and striatal DA fibers
COX-2	MPTP/6-OHDA	MC/CC/VC		Reduced loss of SN DA neurons, striatal DA depletion, and microgliosis
NADPH Oxidase	MPTP		gp91	Reduced loss of SN DA neurons and striatal DA fibers

*PD indicates Parkinson's disease; TNFα, tumor necrosis factor-alpha; TNFR1 and 2, TNFα receptors; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; DA, dopamine; iNOS, inducible nitric oxide synthase; SN, substantia nigra; nNOS, neuronal NOS; 7-NI, 7-nitroindazole; 6-OHDA, 6-hydroxydopamine; L-NAME, N(G)-nitro-L-arginine methyl ester; LPS, lipopolysaccharide; S-MITU, S-methylisothiourea; COX, cyclooxygenase, and MC/CC/VC: meloxicam/celecoxib/valdecoxib.

confer the resistance because deletion of the gene for either receptor alone was ineffective.[102,103] Deletion of the gene for TNFα, on the other hand, afforded only a partial protection against MPTP-induced depletion striatal dopamine and no protection against MPTP-induced loss of SN DA neurons.[104] Furthermore, the degree of neuroprotection conferred by genetic inactivation of either TNFR1 and TNFR2 or TNFα appeared to be dependent on the dosing regiments of MPTP or the severity of DA damage since significant neuroprotection has been primarily observed in the "sub-acute" or "chronic" MPTP PD models[26,102-104] but not the "acute" MPTP model.[104,105] Nevertheless, results from these studies demonstrate that TNFα, through interaction with both of its receptors, contributes significantly to MPTP-induced DA neurodegeneration.

The effect on DA neurodegeneration of gene deletion for IL-1β, which is upregulated in PD brains (Table 2) has not been reported. However, polymorphism in IL-1β gene in humans has been associated with an increased risk and a decreased onset age for idiopathic PD, suggesting a role for IL-1β in the progression of PD.[106-110] Mice lacking the gene for IL-18 (a close relative of IL-1β) whose expression increased concomitantly with the activation of microglia in MPTP-lesioned brains were found to be resistant to the MPTP-induced microglial activation and the degeneration of the nigrostriatal DA pathway.[111] Deletion of the IL-6 gene, on the other hand, rendered mice more sensitive to the MPTP-induced loss of SN DA neurons and depletion of striatal dopamine.[112] This finding is perhaps not surprising since IL-6 may act either as a pro-inflammatory or an anti-inflammatory cytokine.[113] A recent study, however, found no difference between the wild type and IL-6 deficient mice in their vulnerability to MPTP-induced depletion of striatal dopamine.[25] Further studies are needed to clarify the role(s) of these cytokines in the DA neurodegeneration process.

Nitric Oxide Synthase

Mice lacking iNOS gene were less sensitive to MPTP-induced loss of SN DA neurons.[23,24,114] MPTP-induced striatal DA depletion, however, was not affected in iNOS null mice, nor was MPTP-induced microglial activation.[23,24,114] Besides iNOS, the constitutively expressed neuronal NOS (nNOS) may also play a role in mediating MPTP-induced DA neurodegeneration. Mice lacking the nNOS gene were partially protected from MPTP-induced striatal DA depletion.[115] Furthermore, inhibitors of nNOS such as 7-nitroindazole, ARR17338, and s-methylthiocitrulline, which have varying degrees of specificity for different NOS enzymes, afforded significant protection against both MPTP-induced loss of SN DA neurons and depletion of striatal DA in mice and baboons.[115-118] Neuroprotection bestowed by these inhibitors might have involved the interference of additional targets/pathways such as the inhibition of monoamine oxidase B and the prevention of MPTP-induced mitochondrial energy production.[119-122] In addition to the MPTP PD model, pretreatment with a nonspecific NOS inhibitor, N(G)-nitro-L-arginine methyl ester (L-NAME) significantly reduced the depletion of striatal DA and rotations in the 6-OHDA-lesioned animals.[123,124] L-NAME also effectively blocked the amphetamine-induced rotation in the LPS-lesioned animals.[124] L-N(G)-nitroarginine, another nonspecific NOS inhibitor, as well as a selective iNOS inhibitor S-methylisothiourea significantly reduced the loss of SN DA neurons in LPS-lesioned animals.[97,99,100] Findings from

studies using either inhibitors or gene knockout animals demonstrate that excessive production of NO contributes to the progressive DA degeneration in PD. This finding is in line with studies associating polymorphisms in iNOS and nNOS genes with the development of PD in humans.[125,126]

Cyclooxygenase

Similar to the approaches used to determine the contribution of NOS to DA neurodegeneration in PD, inhibitors and gene knockout animals have been used to determine the role of COX enzymes in PD pathogenesis. Mice lacking COX-2 gene, but not COX-1 gene, were significantly less vulnerable to MPTP-induced loss of SN DA neurons and degeneration of striatal dopaminergic fibers.[80,88,127] Inhibitors that had varying degrees of selectivity for COX-2 (meloxicam, celecoxib, or valdecoxib) significantly reduced the loss of SN DA neurons, depletion of striatal DA, and microglial activation in both MPTP and 6-OHDA-lesioned animals.[88,127-129] In addition, NSAIDs such as aspirin, salicylate, and indomethacin significantly reduced the MPTP-induced striatal DA depletion, SN DA neuron loss, and microglial activation.[130-133] The ability of NSAIDs to reduce DA neurodegeneration in experimental PD models is in concert with the reduced incidence of PD observed in NSAID users.[16-18]

NADPH Oxidase

NADPH oxidase is the primary enzyme system responsible for the production of superoxide free radical in a variety of cells including microglia, macrophages, neutrophils, and epithelial cells.[134,135] Oxygen-centered free radicals such as superoxide anion are deleterious to neurons, especially dopaminergic neurons, which are known to be particularly vulnerable to oxidative damage. Moreover, superoxide can react with NO to form peroxynitrite, which is far more toxic than either superoxide or NO.[136,137] NADPH oxidase is a multi-subunit enzyme composed of the plasma membrane-bound gp91 and p22 subunits and the cytosolic p40, p47, and p67 subunits. Upon activation, the cytosolic subunits undergo extensive phosphorylation and translocate to the membrane, where together with small G proteins they associate with the membrane gp91-p22 heterodimer. The assembled and now active enzyme complex then catalyzes the transfer of a single electron from NADPH to molecular oxygen to release superoxide. In mice lacking a functional NADPH oxidase because of the deletion of the gene for the gp91 subunit, significant protection against MPTP-induced loss of SN DA neurons and degeneration of striatal dopaminergic fibers was observed.[90,91] These results demonstrate that the activity of NADPH oxidase is closely related to the dopaminergic neurodegenerative process.

Neuroprotective Agents That Suppress Multiple Pathways of the Pro-inflammatory and Neurotoxic Facade of Activated Microglia

A great many agents have been found to be neuroprotective in various PD models. Showcased in Table 4 are some of the agents selected for their ability to suppress the activation of microglia, inhibit the production of pro-inflammatory and neurotoxic factors, and protect the nigrostriatal DA pathways in various experimental animal PD models.

Minocycline

Minocycline, a derivative of the antibiotic tetracycline has been found to possess anti-inflammatory activity. In the MPTP PD model, minocycline effectively prevented the MPTP-inducing loss of SN DA neurons and degeneration of striatal DA fibers. The neuroprotective effect of minocycline was mediated through its blockade of microglial activation, upregulation of iNOS, and IL-1β, and activation of NADPH oxidase.[89,138,139] Two other studies, however, reported that minocycline enhanced the MPTP-induced degeneration of striatal DA fibers.[140,141] This lack of protection of the striatal DA fibers was attributed to its inability to block TNFα signaling.[25] Nevertheless, in the 6-OHDA PD model, minocycline significantly protected SN DA neurons, reduced microglial activation, and lowered rotation frequencies.[142,143] Furthermore, in the LPS PD model, minocycline markedly reduced microglial and astroglial activation, expression of IL-1β and TNFα, and loss of SN DA neurons.[144] These studies demonstrate that minocycline is capable of inhibiting microglial activation and affording neuroprotection in various PD models.

Vasoactive Intestinal Peptide

Vasoactive Intestinal Peptide (VIP) is an effective anti-inflammatory neuropeptide. Systemic, especially intracranial, delivery of VIP significantly suppressed the MPTP-induced microglial activation, upregulation of IL-1β, TNFα and iNOS, and the loss of SN DA neurons and striatal dopaminergic fibers.[145] In addition to inhibiting the expression of IL-1β, TNFα, and iNOS, neuroprotection afforded by VIP may involve the inhibition of the expression of IL-18 because VIP has been shown to inhibit IL-18 production in human monocytes and deficiency in IL-18 renders mice resistant to MPTP DA neurotoxicity.[111,146] In the 6-OHDA PD model, systemic administration of VIP reduced both the loss of SN DA neurons and the intensities of motor abnormalities.[147]

Peroxisome Proliferator-activated Receptor-γ Agonist

Peroxisome proliferator-activated receptor-γ (PPARγ) agonist is a member of the PPAR nuclear receptor superfamily

Table 4. Effects of Anti-inflammatory Neuroprotective Agents in Experimental PD Models*

Agent	PD Model	On Microglial Activation	On Nigrostriatal DA Pathway
Minocycline	MPTP	(-)† microglial activation, IL-1β, iNOS, NADPH oxidase	Reduce SN DA neuron loss and ST DA depletion
	6-OHDA	(-) microglial activation	Reduce SN DA neuron loss and rotation
	LPS	(-) microglial activation, IL-1β, TNFα	Reduce SN DA neuron loss
VIP	MPTP	(-) microglial activation, IL-1β, TNFα, iNOS	Reduced SN DA neuron loss and ST DA depletion
	6-OHDA		Reduced SN DA neuron loss and motor activities
Pioglitazone	MPTP	(-) microglial activation, iNOS	Reduced SN DA neuron loss
Naloxone	LPS	(-) microglial activation	Reduced SN DA neuron loss
DM†	MPTP	(-) microglial activation	Reduced SN DA neuron loss

*PD indicates Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; IL, interleukin; iNOS, inducible nitric oxide synthase; SN, substantia nigra; DA, dopamine; ST, striatum; 6-OHDA, 6-hydroxydopamine; LPS, lipopolysaccharide; TNFα, tumor necrosis factor-alpha; VIP, vasoactive intestinal peptide; and DM, dextromethorphan.
†(-) indicates reduction or blockade.

of transcription factors activated by ligand-mediated interaction with various hormones and steroids. Among its many cellular actions, PPARγ is an important mediator of the inflammatory process, and its agonists possess potent anti-inflammatory activity.[148] In the MPTP PD model, orally administered pioglitazone, a PPARγ agonist, significantly inhibited MPTP-induced microglial activation, iNOS expression, and loss of DA neurons in the SN. MPTP-induced DA depletion and microglial activation, however, were not significantly affected by pioglitazone.[149,150]

Naloxone

Naloxone is a nonselective opioid receptor antagonist that can interact with all 3 classic opioid receptors (ie, μ-, κ-, and δ-opioid receptors).[151] Association of naloxone with opioid receptors is stereospecific in that only (-)-naloxone is capable of high affinity binding dissociation constant, Kd in the nanomolar range (K_d). Its stereoisomer, (+)-naloxone, on the other hand has very little affinity for opioid receptors (K_d in low micromolar range). However, both naloxone enantiomers have been shown to possess novel anti-inflammatory activity. In particular, both (-)-naloxone and (+)-naloxone can effectively inhibit ROS generation in activated microglia.[152-154] Not only is their inhibitory effect on ROS generation not mediated through the opioid receptor system but they do not possess anti-oxidant activity. In a cell free system used to test anti-oxidant activity, neither naloxone stereoisomer exhibited a significant effect on the reduction of cytochrome c by the superoxide generated in the xanthine-xanthine oxidase system (Figure 3). In the mesencephalic neuron-glia cultures, naloxone isomers significantly protected DA neurons from LPS-induced inflammatory damages.[152] Systemic infusion of naloxone enantiomers dose-dependently inhibited LPS-induced microglial activation and protected against LPS-induced loss of DA neurons in the SN.[31,155] The microglial activity-modulating and neuroprotective property of naloxone isomers did not appear to be limited to LPS-induced DA neurodegeneration. Naloxone isomers effectively protected primary cortical neurons against degeneration induced by either LPS or β-amyloid peptide.[154,156] In addition, naloxone, administered together with an NSAID, indomethacin, effectively reduced LPS-induced microglial activation, depletion of striatal DA, and locomotor abnormalities in rats.[157]

Dextromethorphan

Dextromethorphan (DM) is a nonopioid cough suppressant widely present in a variety of over-the-counter preparations. Although it was considered an N-methyl D-aspartate (NMDA) receptor antagonist, the precise mechanism responsible for its antitussive activity remains unclear. In mesencephalic neuron-glia cultures, DM significantly inhibited LPS-induced microglial activation and production of TNFα, nitric oxide (NO), and superoxide and protected DA neurons against LPS-induced degeneration.[158] In mice, DM significantly reduced the MPTP-induced loss of SN DA neurons.[91] In addition, DM was able to prevent the enhanced loss of SN DA neurons and depletion of striatal DA in a mouse PD model induced by the combination of MPTP and diethyldithiocarbamate, a putative inhibitor of superoxide dismutase.[159] The degeneration of striatal fibers induced by methamphetamine was inhibited by a combination of DM

and MK-801, an NMDA receptor antagonist, possibly through the inhibition of microglial activation.[160]

CONCLUSION

Microglial activation, either as a reactive response to neuronal injuries induced by various neurotoxins, or induced directly by immunologic stimuli appears to play an important role in the degeneration of nigrostriatal DA pathway in PD. Activated microglia, as well as astroglia to a lesser extent, can produce excessive quantities of various pro-inflammatory and neurotoxic factors that include cytokines, free radicals, and lipid metabolites. The participation of microglia in the progressive DA neurodegenerative process in PD seems to occur at a much earlier point than previously thought. This view is strongly supported by findings from epidemiological studies, analysis of postmortem PD brains, determination of the contribution of individual microglia (glia)-derived factors in various PD models, and evaluation of the neuroprotective activity of microglial activity-modulating agents in these models.

In addition to being innately vulnerable to various insults, particularly oxidative damage, DA neurons of the nigrostriatal pathway most likely are residing in an environment that, under pathological conditions, can become particularly hostile. In other words, the midbrain may have a higher abundance of microglia, perhaps being so by design to fend off disturbances and maintain a high degree of homeostasis for DA neurons in the area.[24,42,161-163] However, once becoming activated, by toxin-induced neuronal injuries or immunologic stimuli, those microglia can turn into a robust source of damaging toxic factors to inflict harm to DA neurons in the vicinity. The injuries, in return, will induce additional microglial activation. This self-amplifying cycle of neuronal injury and microglial activation may be one of the key processes that drive the progressive neurodegeneration. This sustainable and accelerating influence may eventually force a significant portion of the DA neurons of the nigrostriatal pathway into demise, leading to the development of symptomatic PD. In this scenario, microglia, presenting their deleterious facade of being pro-inflammatory and neurotoxic, play the role of the accelerator of DA neurodegeneration at a rather early stage of the progressive process. Dysfunctions in the DA neurons themselves including improper protein folding/disposal, mitochondrial impairment, and oxidative damage, either as a result of direct exposure to specific toxins or as a consequence of the occurrence of a damaging genetic event, or both, play the role of the initiator of DA neurodegeneration (Figure 4).[164-167] Therefore, the validity and potential of targeting microglial activation to break the vicious self-propagating cycle to modify the course of neurodegeneration, especially at a stage long before the appearance of clinical symptoms, can be enormous, as exemplified by the various neuroprotective

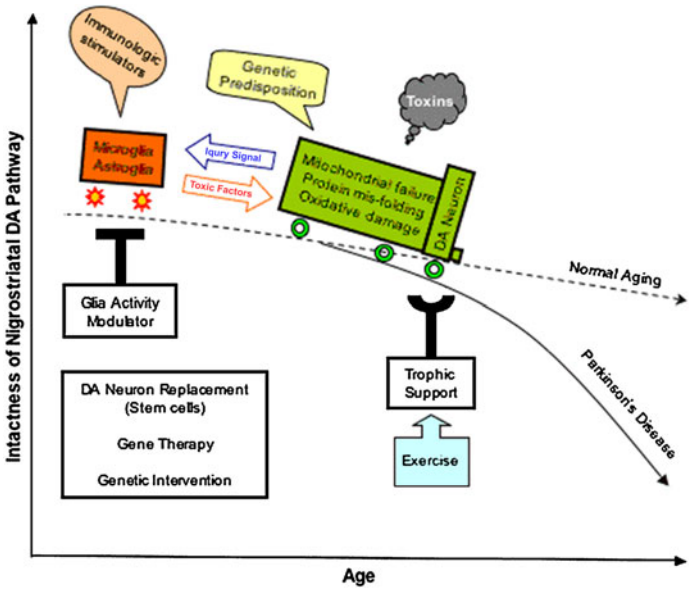

Figure 4. Schematic representation of the microglial activation-accelerated DA neurodegeneration and potential neuroprotective strategies. DA indicates dopamine.

studies. At a minimum, microglial activation-modulating treatment can well be a part of a multi-prone therapy for PD that will include enhancement of trophic support for DA neurons through exercise or gene delivery, repopulation of DA neurons with genetically engineered cells or stem cells, and genetic correction of defects that are yet to be discovered in idiopathic PD (Figure 4).[168-170]

PERSPECTIVE

The goal of neuroprotection is to slow down, or better yet to stop or even reverse the progressive neurodegenerative process. Several issues need to be considered in designing future microglial activity-modulating neuroprotective studies.

First of all, multiple animal models currently exist for PD. Most of them reproduce certain features of the disease and perhaps none of them is an all-encompassing animal-PD model. This issue has certainly complicated the validation process for neuroprotective agents for PD. Therefore, neuroprotection of a given agent may need to be validated in more than one PD model. Second, it probably will not be sufficient to block the action of individual microglia (glia)-derived factors to effectively alter the course of neurodegeneration. Combinational therapy using a cocktail of various microglial activity-modulating agents may be needed to offer a satisfactory and sustained level of neuroprotection. Third, activated microglia produce more than pro-inflammatory and neurotoxic factors, they also produce trophic factors. In addition, microglia are needed for immune surveillance and tissue repair. Therefore, a complete shutdown of microglial activity is certainly not desirable. Agents that specifically modulate the pro-inflammatory and neurotoxic

activities of microglia would be the top choice for further development. Finally, the side effects and toxicity of the agents, especially over long-term use for the management of a chronic disease such as PD, are of particular concern. Therefore, agents that have a previous track record of safety would be good candidates for further evaluation, and they can serve as good templates for the development of even better drugs for the treatment of PD.

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